

WE CLAIM:

1. A method of constructing a grid for use in aligning two or more images, where said images reflect corresponding arrays of signals, comprising:
applying an algorithm to the array which finds the shortest paths among the local
5 intensity minima so as to delineate the local intensity maximum associated with a signal in the array.
2. The method of claim 1 wherein the array of signals originate from an array of encoded beads, and said encoding is by a detectable signal.
3. The method of claim 1 wherein the algorithm is Dijkstra's "shortest path"
10 algorithm.
4. The method of claim 1 wherein the algorithm finds the grid as an optimal path as follows:

 computing the external gradient image by subtracting a dilated image from the original image;

15 determining overall orientation by: (i) establishing horizontal and vertical reference lines, (ii) computing the shortest paths along two directions, (iii) computing a ratio by dividing each shortest path length by the length of the corresponding reference line; and (iv) selecting the reference line yielding the ratio closest to unity to determine the overall orientation;

20 finding the horizontal grid partition by shifting reference line by one grid unit to a new position and computing the shortest path, and continuing said shifting and computing until the shifted reference line falls outside the array boundary;

finding the vertical grid partition by finding the shortest path along a diagonal direction, and computing intersections of this diagonal path and every horizontal partition, provided that, given the intersections of the diagonal partition and two consecutive horizontal partitions, the vertical partition will be located at the midpoint
5 of these intersections.

5. The method of any of claims 1 to 4 wherein following construction of the grid, the grid is grown or shrunk to the expected array boundary.

6. The method of claim 5 wherein grid stagger is corrected.

7. The method of any of claims 1 to 4 wherein the grid coordinates are stored in a
10 file.

8. The method of claims 1 to 4 wherein the algorithm is applied by a programmed computer.

9. A method of determining alignment between two or more images, where each image is of a correspondingly arranged array having corresponding subsets of
15 distinguishable signals, comprising:

establishing a two-dimensional grid having units, each unit corresponding to a particular position in the arrays from which a signal originates, and aligning the two grids such that corresponding signals align in a one-to-one correspondence such that a detectable difference results when one array is shifted out of alignment by one or
20 more grid units with respect to the other array.

10. The method of claim 9 wherein the detectable difference results from variation in the correspondence of one or more of the subsets.

11. The method of claim 10 wherein the signals are optical signals and the additional signals can be seen by viewing the arrays in another color channel.

12. The method of claim 9 wherein different signals in the array originate from beads, and different beads occupy different positions in the array.

5 13. The method of claim 12 wherein the array of beads includes differently colored beads, and the colors are detected.

14. The method of claim 9 performed by carrying out the steps of the following pseudocode:

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LoadDecodingData(DecodingDataRecord, DecodingMap);
10 LoadAssayData(AssayDataRecord, IntensityArray);

MinVariance = -1000;
MinVarianceLocation = 0;

15 /** Check Variance Produced by 7 Possible Unit Displacements - see Text */
For (i=0; i< 7; i++){
    SiftGridPosition(i);
    FilterDarkBeads();
    Variance[i] = Merge(DecodingMap, IntensityArray);
20
    IF(Variance[i] < MinVariance){
        MinVariance = Variance[i];
        MinVarianceLocation = i;
    }
25 };

WriteAssayData(MinVarianceLocation, AssayDataRecord); /** Save Location */
```

15. The method of claim 14 wherein the pseudocode is performed by a programmed
30 computer.

16. A method of determining alignment between two or more images of correspondingly arranged arrays having corresponding colored components, wherein

some of the components are differently colored, without reference to a brightfield image, comprising:

constructing horizontal and vertical intensity profiles by respectively projecting image intensities to the top-most and left-most scan line in the display;

5 evaluating the intensity variation in each profile;

rotating the color image by a pre-defined angle and repeating the constructing and evaluating steps in the new position;

continuing to rotate until the profiles exhibit maximal variations; and

reducing the step size in rotation angle and reversing the direction of rotation until

10 the optimal rotation angle is found.

17. The method of claim 16 wherein the components are beads.

18. The method of claim 16 wherein, the array geometry is known before practicing the method, and the angle of maximal variation is obtained from the array fitting.

19. A method of image segmentation producing a partition of an image of a planar

15 array of objects, comprising:

determining overall orientation of the array using a set of reference lines which are brought into alignment with symmetry axes of the array;

replicating the reference lines by shifting normal to each symmetry direction and

finding the shortest path connecting the starting point and point of each shifted

20 reference line;

replicating the replicating step until the replicated reference line falls outside the array boundary.

20. The method of claim 19 wherein the overall orientation is determined using horizontal and vertical reference lines of known length and computing the shortest path from the starting and end point of each reference line, the path following the local intersection minimal path.
- 5 21. The method of claim 20 wherein the minimal path is the path of shortest length following the locus of local intensity minima.
22. The method of claim 19 wherein the partition is in the form of a mesh, each mesh field delineating one object.
23. The method of claim 19 wherein objects are color-encoded beads functionalized with receptors capable of binding ligands contained in an analyte solution.
- 10 24. A method of generating a decoding map by constructing constituent clusters in accordance with a decoding map template providing one seed location for each cluster, each member of a particular cluster having the same identifying characteristic, the method comprising:
- 15 providing a set of seed locations with one seed location for each anticipated cluster; constructing from a first scatter plot of the array members a two-dimensional histogram of clusters; producing a partition of the histogram; and growing clusters around each seed position to thereby generate a decoding map.
- 20 25. The method of claim 24 further including the step of constructing an additional scatter plot of the array members, where said additional scatter plot is constructed from monitoring a different identifying characteristic than is the first scatter plot.
26. The method of claim 24 wherein the identifying characteristic is color.

27. The method of claim 26 wherein the different identifying characteristic is a different color from the color used for constructing the first scatter plot.

28. The method of claim 24 further including the step of smoothing the two-dimensional histogram.

5

29. A method of autocentering for viewing under a microscope, a rectangular image of a rectangular array of coding signals, comprising:

providing the image in the viewing field of the microscope;

ascertaining the number of sides and the number of right-angled corners of the

10 image, and if said numbers indicate that a rectangular image is in the viewing field,

arresting movement of the field, but where said numbers indicate that a non-

rectangular image is in the viewing field, moving the viewing field such that a

rectangular image is captured in the viewing field.

30. The method of claim 29 wherein the array of coding signals is an array of

15 encoded beads with receptors associated therewith.

31. The method of claim 30 wherein at least one of the receptors is associated with a

ligand, and the ligand is associated with a second detectable signal, different from

said coding signals.

32. The method of claim 29 which is effected by applying the following

20 pseudocode:

OpenImg(InputImg, OpenedImg); /** apply sequence of morphological erosion
and dilation operations to eliminate internal structure of the image showing particle
array **/

25

Binarize(OpenedImg,BinImg);

/** apply optimal thresholding

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algorithm **/.

CloseImg (BinImg, ClosedImg);           /** apply sequence of
morphological dilation and erosion operations **/

5 AnalyzeConnectivity (ClosedImg, ConComp);           /** find connected
components in closed image **/

Filter (ConComp, FilteredConComp);           /** filter out all “non-
10 box-like” regions; a “box-like” region is defined as a region whose area is close to
the area of its “bounding box” **/

Center = FindMaxConComp(FilteredConComp); /** find largest connected
component that is smaller than 70% of the image size and find its centroid **/
15 MoveXYTranslator ( Center.X, Center.Y );           /** position stage **/.

33. The method of claim 32 wherein the pseudocode is applied by a programmed
computer.
20

34. A method of conducting and analyzing assay data of patient samples at different
locations, comprising:

obtaining a patient sample;

conducting an assay on the patient sample using a uniquely encoded array of
25 receptors where the assay involves contacting the array with the patient sample, and
determining if there is association between any of the receptors and ligands in the
patient sample based on signals from receptors in the array;

decoding the array to determine the receptors associating with ligands and thereby
provide assay results;

30 wherein the decoding step is conducted at a different location from the other steps,
and an image of the uniquely encoded array recorded before or after conducting the
assay is provided to said different location.

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35. The method of claim 34 wherein the uniquely encoded array is an array of encoded beads, wherein different beads have different receptors associated therewith.
36. The array of claim 35 wherein the encoding of the beads is with different colors.
37. The array of claim 35 wherein the signal from the receptors results from the association of a fluorescent entity with the ligand attached to the receptor.
38. The method of claim 34 wherein the assay is conducted at a different location from where the patient samples are obtained.
39. The method of claim 34 further including the step of providing an image of the uniquely encoded array recorded before conducting the assay to the site where the assay was conducted, to the patient, or to another site.
40. The method of claim 34 further including the step of providing an image of the uniquely encoded array recorded after conducting the assay is provided to the site where the assay was conducted, to the patient, or to another site.
41. The method of claim 34 wherein the assay relates to patient genetic information or to information about certain proteins or antibodies in a patient.
42. The method of claim 34 further including the step of transmitting the images to a site where the images are archived.
43. The method of claims 34 or 42 wherein the identity of the patient providing the sample and assay images and results are available only to authorized parties.
44. The method of claim 43 wherein all images are transmitted with an encryption key, so that an image can only be accessed by an authorized party with access to the encryption key.

45. The method of claim 44 wherein the encryption key is a pattern of the encoded beads.

46. The method of claim 44 wherein the encryption key is a bar code or other unique identifier code.

5 47. An encrypted array of receptors, where at least some of said receptors are predicted to associate with ligands in a patient sample, comprising:
an array identifying code physically associated with the array; and
encoded identifying oligonucleotides physically associated with the array, said oligonucleotides being predicted to associate with particular oligonucleotides derived
10 from the patient sample, wherein, following such association and decoding of the oligonucleotide encoding, an oligonucleotide code is provided which allows identification of the patient from which the sample was derived.

48. The array of claim 47 the array identifying code is a bar code affixed to a substrate to which the array is affixed.

15 49. The array of claim 47 wherein the array identifying receptors are associated with the array by affixing them to a substrate to which the array is affixed.

50. The array of claim 47 wherein the array includes encoded beads which can be decoded to indicate the type of receptor which is affixed thereto and where the beads are affixed to a substrate.

20 51. The array of claim 50 wherein the array identifying code is produced by a unique pattern of the encoded beads on the substrate.

52. The array of claim 47 wherein said identifying receptors are oligonucleotides.

53. The array of claim 52 wherein the oligonucleotides are DNA.

54. The array of claim 52 wherein the identifying oligonucleotides are affixed to encoded beads, which can be decoded to indicate the identifying oligonucleotide affixed thereto and where the beads are affixed to a substrate.
55. The array of claim 52 wherein the identifying oligonucleotides are derived from
5 a highly polymorphic region of a patient's genome.
56. A database comprising arrays of encoded beads bound to different receptors and wherein the beads are affixed to a substrate, some receptors being associated with ligands derived from patient samples, where the substrate has affixed thereto an array identifying code, and wherein the substrate further includes encoded beads bound to
10 oligonucleotides affixed thereto, some of which are predicted to associate with oligonucleotides derived from a patient sample, such that, following such association and decoding of the encoded beads, a patient code is formed permitting identification of the patient from which the sample was derived, and wherein information relating to an array in the database can be accessed only by parties possessing the patient
15 code associated with that array.
57. The database of claim 56 wherein the pattern of the encoded beads forms an array identifying code.
58. The database of claim 56 wherein a bar code affixed to the substrate is the array identifying code.
- 20 59. The database of claim 56 wherein the database can only be accessed by parties further possessing the array identifying code.
60. The database of claim 56 wherein the substrate has a planar surface to which the beads and the encoded beads are affixed.

61. The database of claim 56 wherein information relating to an array includes an image of the array permitting decoding of the encoded beads.
62. The database of claim 61 wherein the image permits identification of the associated ligands.
- 5 63. The database of claim 56 wherein the database can only be accessed by parties transmitting the patient code to the database.
64. A method of ensuring confidentiality of information from assays of patient samples such that only an authorized party can determine the assay results for an identified patient, and also of ensuring that assay information is properly associated
- 10 with the source patient, comprising:
- physically associating an assay identifying code with an assay;
 - physically associating an assay with encoded identifying oligonucleotides which are predicted to associate with particular oligonucleotides derived from a patient sample, wherein, following such association and decoding of the encoding, an
 - 15 oligonucleotide code is provided which allows identification of the patient from which the sample was derived;
 - assaying a patient sample with the assay at a testing center;
 - transmitting assay results to an authorized party if and only if the authorized party provides the oligonucleotide code.
- 20 65. The method of claim 64 wherein the testing center is not aware of the patient's identity.
66. The method of claim 64 wherein the testing center provides information about the assay to an information keeper.

67. The method of claim 66 wherein the information keeper is not aware of the patient's identity.

68. The method of claim 64 wherein the assay identifying code is a bar code affixed to a substrate on which the assay is conducted.

5 69. The method of claim 64 wherein the assay identifying code is formed by an encoded array on the surface of a substrate on which the assay is conducted.

70. The method of claim 64 wherein the information is transmitted only if the array identifying code is also provided to the information keeper.

71. The method of claim 64 wherein the encoding of the identifying
10 oligonucleotides is not provided to the testing center.

72. The method of claim 64 wherein the assay is performed by monitoring association between an array of encoded receptors (which form the assay identifying code) with ligands in the patient sample and wherein the assay identifying code is not known by the testing center but is known by the information keeper.

15 73. The method of claim 72 wherein the oligonucleotide code and the assay identifying code are provided to the patient by the information keeper.

74. A method of blinding a multiplexed assay where the party conducting the assay does not know the results, and also of ensuring that assay information and results remain associated with the sample which is assayed, where the assay involves
20 contacting a sample with an array of encoded receptors wherein a ligand in the sample is predicted to associate with a receptor in the array, and wherein the encoding of the array and the assay results can be imaged, comprising:
physically associating a sample identifying code with the array of encoded receptors;

providing the encoded array (without providing the encoding) and having the party provided the encoded array conduct the assay on the sample using the encoded array; transmitting an image of the results of the assay to a party with the encoding; and decoding the image.

5 75. The method of claim 74 wherein the receptors are associated with encoded beads, such that the encoding permits identification of the bead associated with a receptor.

76. The method of claim 75 wherein the encoding is by color.

77. The method of claim 75 wherein the beads are placed on a substrate and the
10 sample identifying code is associated with the substrate.

78. The method of claim 75 wherein the receptors are proteins including antibodies, oligonucleotides, or other organic or inorganic compounds.

79. The method of claim 75 wherein the sample is predicted to contain a protein including an antibody, oligonucleotide, or other organic or inorganic compound.

15 80. The method of claim 74 wherein the sample is derived from a patient and a patient identifying code is associated with the array of receptors, and the assay results are only provided to parties providing the patient identifying code.

81. The method of claim 80 wherein the patient identifying code is an array of encoded identifying oligonucleotides which are predicted to associate with particular
20 oligonucleotides derived from a patient sample.

82. An integer representation of the configuration of a multi-constituent planar array of L encoded beads wherein there are n distinguishable types of beads, the code having $Q_1, Q_2, Q_3, Q_p \geq n$ elements, and wherein each bead is assigned a $(R+1)$ -tuple of integers in which one field represents the position index, l , where $1 \leq l \leq L$,
5 and each of the remaining fields represents an exponent, x , where $1 \leq x \leq Q_p$.
83. The integer representation of claim 82 wherein if $P=3$ and $Q_1 = Q_2 = Q_3 = Q_p$, then there is a corresponding r, g, b code having Q^3 elements and the 4-tuple having the form (l, r, g, b) where the integers r, g, b each assume a value in the range $0 \leq r, g, b \leq Q$.
- 10 84. A method of encrypting the identity of a type of receptor, among multiple receptor types, which is attached to particular encoded carriers in an array of encoded carriers, comprising:
encoding the carriers using a multi-component code;
uniquely associating a type of receptor with carriers encoded with one component of
15 the code.
85. The method of claim 84 wherein the code is a multi-color code.
86. The method of claim 84 wherein the receptor is an oligonucleotide or a protein.
87. The method of claim 85 wherein the colors are formed by mixing differing ratios of more than one color.
- 20 88. The method of claim 85 wherein the code is formed by combinations of more than one color.
89. The method of claim 84 wherein the carriers are beads.

90. A method of generating a two-dimensional decoding map of a scatter plot of an array of signals, where the decoding map can be used to decode a corresponding array of signals, comprising:
- converting a scatter plot it to a distance graph image;
- 5 processing the distance graph image by applying a morphological Open operation to each connected graph such that the steps of erosion and dilation constituting the open operation will alter the graph node values;
- eliminating, for each node in turn, all edges whose weight exceeds the node's new value;
- 10 partitioning the graph into connected components, wherein a connected component or cluster is a sub-graph of connected nodes and each node within a connected subgraph can be visited by traversing edges; and
- filtering out small groups and splitting large groups into two groups, if necessary.